"Immunomodulatory Effect of Selected Fruit Waste by Product Leads on Cyclophosphamide Induced Immunosuppression in Mice"

Research Proposal Submitted in Partial Fulfillment of the Requirements for the Degree of

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Humans inhabit an open environment that is extensively polluted with several toxins. They are frequently exposed to a wide range of environmental, chemical, and physical variables that harm and harm human beings. Our bodies naturally employ a variety of strategies to manage the aforementioned concerns, but man is continuously looking for and identifying solutions to these issues. Plants have been utilised to treat a variety of human problems from very early times. There are claims that some plants may treat ailments including AIDS, cancer, and diabetes.[1]

As old and widespread as medicine itself is the use of plant-based medications for health. Natural ingredients were the cornerstone of all medications till the turn of the century. Herbalism still exists in the developing world even if it has diminished in the West. WHO estimates that more than 70% of the world's population still uses herbal treatments to treat their medical needs.[2]

There are a tonne of chemical compounds originating from plants that are used today in one or more nations throughout the world and are regarded as major medications. Numerous plant compounds influence the immune system in some way. They either boost immune response to aid in the body's removal of unwanted substances or they reduce immunological response to stop the body from deteriorating. Immuno modulatory agents are substances that change immune response.[3]

Similar to how our armies defend our nation, our immune systems defend our bodies from thei nvasion of alien organisms. It never stops being proactive and has the capacity to tell "self" from "nonself." It goes without saying that those with immune responses below the "normal" th reshold are more prone to infectious diseases.

The quality of one's diet plays a major reinfesting immunological competence. It is crucial for the production of free radicals, cell

proliferation, immune system inhibition, and the creation and release of signalling molecules. Our immune system's functioning might be disturbed by undernutrition or dietary deficiencis, which increases our susceptibility to many illnesses.[4]

External defences

Physical barriers- An attachment of bacterium to the surface of epithelial layers of host is a critical step before its entering in the host body and thus causing a disease. Both active and passive processes are used by organisms to enter the body. For instance, they can pierce the skin, eat their way into food, enter the respiratory system by inhalation, and also through a

wound area, since much of the targets the respiratory tract to enter in the host body and further their survival.

Regardless of their site of entrance, these parasites are impeded by various layers, such as the dead layer of skin and also the epithelial cells covering the cavities that come into touch with the outside, including the respiratory, genitourinary, or gastrointestinal tracts. In actuality, these tracts serve as the primary entrance point for microorganisms entering the body.[5]

Secretions-

Secretion variations at epithelial surfaces play a crucial role in defence. The main goal is to create an environment that is unfavourable to microbial colonisation. Some compounds, such as lysozymes, which break down proteloglycans in bacterial cell walls, are known to directly kill germs. Other molecules compete with microbes for nutrients (such as transferring iron), while still others disrupt ion transport (e.g., NaCl).

The mucosal epithelial cells release mucus, which contains mucin, coating their surfaces and preventing microorganisms from attaching to them, which is a need for access into the body.

Urine, saliva, and tears all operate as washers, preventing bacteria from adhering to epithelial surfaces. Additionally, IgA antibodies released by epithelial cells also impedes the adhesion of various microorganisms, reported to be present in saliva and tears. The genitourinary tract also contains these antibodies. Numerous tiny peptides with strong anti-bacterial activities (peptide antibiotics) are also known to be produced by phagocytes, respiratory epithelia, and other bodily tissues.[6]

Bacterial products and competition-

Normal commensals (non-pathogenic microorganisms) aid in infection prevention as well. These non-pathogenic bacteria are present in the mouth, on the skin, and in the gastrointestinal and reproductive tracts. Billions of bacteria having symbiotic harmony with host have been reported in the digestive system. These bacteria aid in stopping the spread of diseases by producing short-chain fatty acids and colicins, which are antibacterial proteins.[7]

The Immune System-

Vertebrate immune systems are very complex adaptive defensive mechanisms that keep them safe from infectious diseases and cancer. It can produce different kinds of cells and chemicals that can identify and get rid of a huge variety of foreign invaders. The immune system's two interconnected functions of recognition and reaction make up its functional division. The immune system has the ability to distinguish between foreign molecules and host one's cellular components. When a foreign component is detected by the immune system, it reacts by using the initial recognition data to destroy or neutralise it. If the immune system comes into contact with the same antigen again, the same reaction could occur (memory response)[8]

Host immune system is a sophisticated collection of leukocytes, other blood components and antibodies that both defends against external intruders and also preserves self-tolerance. Additionally, a variety of specialised stromal and epithelial cells offer the anatomic environment that controls the immune system's varied tasks by secreting a number of essential substances. Various cell types, tissues, and organs are composing the immune system which collaborate all these to mainly protect the body from "external" intruders. Among these populations, various microbial populations including bacteria, viruses, parasites, and fungus are some common examples. Among these most of them are known to have parasitic inhabitancy in the host, thus immune system's function is the critical surveillance of these populations to avoid their entrance [9]

Host immune system is comprised of various immune cells and their associated organs forming a network which protect the host from various illness. Immune deficiencies, cancer, inflammatory illnesses, and autoimmune diseases can all be brought on by immune system disorders. By interfering with its functioning, immunomodulation can change an organism's immune system. If the method boosts an immune response, it is referred to as an immunostimulative medication, which usually refers to the stimulation of non-specific immune system. Immunosuppression entails mainly lowering resistance to stress, infections, and other conditions, and it may be brought on by environmental or pharmacologic causes.

To control the regular immune functioning, one must take into account both immunosuppression and immunostimulation. Since immune stimulating and immunological depressing medicines each have a distinct function, research into more effective agents that can perform these functions is now a primary focus on a global scale.

Numerous 'Rasayana' and Indian medicinal herbs are said to have immune-modulating properties. Using plant-based immunomodulators are also the popular method for protecting the host whereas various polysaccharides, lectins, peptides, flavonoids, and tannins have been identified to having immunomodulatory functions. Traditional medicine-based administration of plant extracts has been used to treat a variety of ailments since ancient times. Immunosuppressive and immunostimulative agents include natural adjuvants, synthetic substances, and antibody reagents. However, there are significant drawbacks to using these drugs widely, including a higher risk of infection and a widespread impact on the immune system.[10]

Organ involved in Immune System-

Primary lymphoid Organ-

T-cells and B-cells, which are produced by the thymus gland and bone marrow, are sent to secondary organs for maturation, proliferation, and storage through lymphatic channels. Blood cells are formed and differentiated in the bone marrow, which is a soft tissue present in the bone cavities, from where leukocytes also arouse by stem cell population. B and T lymphocytes are produced from lymphoid cells in the bone marrow and hereafter matured in spleen and thymus. In the bone marrow, B-cells develop and undergo differentiation before being sent to the various locations.

Secondary Organs-

Various secondary organs such as lymph nodes, spleen, tonsils, and Peyer's patches (liver), appendix, small intestines, and tonsils are the areas where the immune components mature and reside in order to combat with foreign components [11].

Specialized Cells of the immune system-

Leucocytes-

White blood cells, or leucocytes, are the primary immune system cells that offer either innate or particular adaptive immunity. They have particular roles and are motile. In normal blood, there are between 4,500 and 11,000 leucocytes per millilitre. The majority of leucocytes are not in the bloodstream and those that are are moving between different sites. Additional divisions include granulocytes, monocytes, and lymphocytes. The most abundant leucocytes play a crucial role as inflammatory response mediators. Neutrophils make up between 50% and 70% of granulocytes, followed by eosinophils at 4% and basophils at 5%.

Neutrophils-

Neutrophils are a type of white blood cell leading majorly for host immune response, where as there also other four types of cells in white blood cells category. Among the all types of WBCs, neutrophils constitute maximum population of WBCs, between 55-70% of total white blood cells. As WBCs are also known as leukocytes, and also known to be the key component of immune system. The size of human neutrophils ranges from 9-15 nm where the nucleus having two to five distinct lobes connected by filaments resembling to hair like structures. As a part of complex immune system, where white blood cells remained in your bloodstream and lymphatic system. In sick condition or having an injury, your immune system becomes active to identify the foreign antigens, and their removal by various immune responses. Various chemicals produced by the white blood cells are taking part in these immune reactions, as a part of inflammatory responses towards an infection. Neutrophils are also vital since having

limited localization in the circulation. These are freely movable through the walls of veins and thus moving in tissue areas for the immediate attack on nearby antigens [12].

Eosinophils-

Among the types of white blood cells, eosinophils are those having important role in disease fighting. Among these diseased conditions, parasitic infections, allergic reactions or metabolic disorders such as cancer are the most common. If you having high levels of eosinophils in your blood or in tissues, upon an infection causing inflammation in nearby areas, then your counts will be high calling eosinophilia.

These cells having granules (small particles) with enzymes which released upon infections,particular to allergic reactions, and asthma. In a healthy individual, eosinophils counts for about 1–3% of total white blood cells. These are about 12–17 micrometres in size and having a bilobed nuclei.

Basophils-

Heparin and histamine are found in the numerous big cytoplasmic granules that are present. They produce histamine and other mediators involved in allergic responses when agitated. Additionally, they make cytolcines. Basophils exhibit Ig E surface membrane receptors with great affinity.

Monocytes-

The bone marrow also produces monocytes. Up to 10% of the blood's leucocytes are made up of them. The majority, however, travel into practically all tissues and exit the circulation within a few hours to become macrophages.

Highly adherent, mobile, and phagocytic cells characterise both monocytes and macrophages. They coordinate and control other immune system cells. They function as cells that process and deliver antigens and, when equipped with certain IgG antibodies, they become cytotoxic cells. [13]

Natural killer (NK) cells-

Natural killer cells are immune cells and part of primary immune response which protect the host from various immune stimulations, thus calling as "natural killer cells". NK cells do not have antigen-specific receptors as B and T cells do. They are an essential component of innate immunity and aid in the destruction of tumours and virus-infected cells. The azurophilic granules in their cytoplasm, which are a distinguishing feature of NK cells that are generated from bone marrow, help identify them. In addition to natural killer cells mediated cytotoxicity, these cells causing antibody-dependent cellular cytotoxicity (ADCC), by specific binding between Fc region of IgG antibodies. NK cells composing vary little portion of lymphoid cells in the spleen and also for other secondary lymphoid organs which are particular to peripheral

blood cells. No antigen-specific receptors are present on NK cells. During their interactions with MHC molecules, these are able to block the cytotoxic functions by specifically blocking the receptor localized on these MHC molecules. Thus, they eliminate self-cells whose expression of class I molecules has been downregulated. When target cells are treated with IFN-, MHC class I expression rises. sAs a result, there are often opposing needs for TCTL and NK cells when targeting cells. The growth or spread of a number of microbial illnesses has been found to be significantly restricted by NK cells, in addition to their antitumor activity. Through the production of a number of cytokines, NK cells may also have an immunoregulatory function in controlling haematopoiesis.

Lymphocytes

These are the specific cell types mainly found in the lymphoid organs, thus called as lymphocytes, whereas these are known for their specific functions for capturing the antigens and their removal from the lymph fluid, are where they enter the systemic circulation. About 25–50% of the blood's leucocytes are lymphocytes.

They are following type B, T lymphocytes

Need For the study

Our immune system having the various protective mechanism particular to host pathogen interactions, which are basically the part of host defence mechanism. Interestingly, an alterations or non specific augmentation of the immune responses are also lethal and toxic in nature causing onset of hyper inflammation and non-immune chronic inflammatory diseases, such as allergies, rheumatoid disorders and some others. Having the variable degree of effectiveness, various immune drugs particularly the organic synthetics, and immune components such as cytokines and antibodies specifically targets various pathways found to be critical in immune-related illnesses. In actual practise, these immune drugs and stimulants having serious side effects and health issues, whereas treating immune-related disorders with herbal medicines have been found to be interesting, in order to be away from the side effects of various immunosuppressants and immunostimulants. The extensive range of immunomodulatory actions and influences that plant extracts have on the human immune system are thought to be the cause of many of the therapeutic benefits that have been associated to them. Various phytomolecules including alkaloids, diterpenoids, glycosides flavonoids, polysaccharides, and lactones have been found to having significant immunomodulating effects. Therefore, in order to identify safe and effective medications as immunosuppressant and immunostimulant properties, the present study has been proposed with below mentioned objectives and details.

Aim and Objective

Aim- Immunomodulatory Effect of Selected Fruit Waste by Product and leaf, Leads on Cyclophosphamide Induced Immunosuppression in Mice

Objective-

- Identification and preparation of extract of abundantly available fruit waste by products and leaf
- In vitro anti-inflammatory activity of fruit byproducts and leaf on LPS induced inflammation in peritoneal macrophages
- Cell cytotoxicity profile of potent lead molecules using standard bioassays
- In-vivo molecular mechanism of potential leads for immunomodulatory activity with special emphasis on inflammation pathway
- In vivo toxicity profile of potent leads
- Chemical signature profile lead using chromatographic techniques

Literature Review

- In a study by ***Y. Wu et al. (2019)*** pomegranate peel polysaccharides (PPP) have been found to have significant immunosuppressive effects in mice where the cyclophosphamide (CTX) was used as an immunostimulant. Mice were randomised into 6 groups and study period was for a total of 28 days, PPP was administered by oral gavage at the doses of 100, 200, 400 mg/kg bd. wt. The received data clearly suggest that PPP caused significant restored the loss of body weight of the immunosuppressed mice and also raised their immunological organ indexes to normal levels. As PPP was administered to mouse spleens by gavage, it significantly increased the LDH (lactate dehydrogenase) and ACP (acid phosphatase) activity, as compared to controls animals. In cyclophosphamide-induced immunosuppressed mice, PPP promoted splenic lymphocyte proliferation and secretion. It also significantly enhanced the expression of immunoglobulins (Ig-A, Ig-G, and Ig-M) and release of various cytokines (TNF-, IL-2, and INF-). Mice treated at the higher doses of PPP exhibited enhanced enzymatic activities particularly, the hepatic antioxidant enzymes such as T-AOC (total antioxidant capacity), T-SOD (total superoxide dismutase), GSH-PX (glutathione peroxidase), and CAT (catalase). In conclusion, PPP can be employed as an effective adjuvant immunopotentiating treatment, a substitute for reducing chemotherapy-

induced immunosuppression, and an immunostimulant for the food and pharmaceutical sectors.[16]

- **H. Ahmadi Gavlighi et al. (2018)** have extracted the PPP extract with 0.1 M HCl, Cellic CTec2, and buffer, the molecular features, structural characteristics, and studied immunostimulatory properties of PPP derived polysaccharides. The neutral sugars and uronic acids that made up the separated polysaccharides were the principal building blocks, with varied amounts of proteins also present. The structure of isolated polysaccharides was built from a variety of sugars, including glucose, galactose, mannose, arabinose, and rhamnose. Polysaccharides varied greatly in average molecular weight (Mw), ranging from 422.5 103 to 18,631.8 103 g/mL. Nitric oxide and cytokines such as IL-1, TNF-, IL-6, and IL-10 were significantly observed in RAW264.7 murine macrophage cells culture supernatant, in response to polysaccharide polymers made utilising the Cellic CTec2 enzyme. Upon the activation of various transcription factors (p-NF-B, p-JNK, p-ERK, and p-p38 proteins) as well as the NF-B and MAPK signalling pathways, macrophage cells were stimulated by polysaccharide. The most effective immunostimulatory polysaccharides having branching points at C6 and C3 and are composed of (1 3)-linked glucose, (1 6)-linked galactose, (1 4)-linked mannose, and (1 4)-linked arabinose residues. These findings are suggestive for the identification of key structural factors responsible for stimulating macrophage cells mainly due to a decrease in the molecular weight of various polysaccharides isolated with Cellic CTec2 enzyme.[17]
- **Miodrag Coli´c et.al (2022)** They studied ellagitannins may be the reason of the considerable immunomodulatory action that pomegranate peel extract (PEx) displayed. The aim of this study was particular to the identification of various constituents of ellagitannin having the ability to modulate cytokine production. Methods: After being stimulated with phytohemagglutinin, peripheral blood mononuclear cells (PBMCs) were treated with varying doses of PEx or punicalagin (PG), punicalin (PN), and ellagic acid (EA). These treatments were performed in alone or in combination with standard drugs. We measured cytotoxicity, cell growth, and cytokine generation. Results: All chemicals significantly reduced the cell growth at non-cytotoxic doses. IC_{50} values of (mg/mL) EA (7.56), PG (38.52), PEx (49.05), and PN (69.95) have been recorded. PEx and all ellagitannins suppressed the expression of TNF-α, IL-6, and IL-8 levels in a dose-dependent manne. Additionally, their combinations have also exhibited synergistic effects. Lower concentrations of PEx and EA promoted the expression of IL-10 (Treg cytokine), whereas higher amount of PEx and ellagitannins suppress Th1

and Th17 immune responses. However, unlike PEx, neither ellagitannin component boosted Th2 response. Conclusions: Without having a substantial synergistic down-modulatory impact on T-cell cytokines, various combinations of PG, PN and EA enhanced anti-inflammatory responses. Interestingly, ellagitannins were not found to have stimulatory impact of PEx on the Th2 response, although the enhanced production of cytokines such as IL-10 have been observed with PEx treatment, which might be due to EA [18]

- **Miodrag Coli´c et.al (2022)** reported the anti-inflammatory and antioxidant characteristics of pomegranate peel extract (PoPEx). Although limited reports are available regarding the mechanistic evidence for these properties. In this work, effect of PoPEx (range 6.25–400 g/mL) extract was studied on peripheral blood mononuclear cells (PBMC) derived from human which led to cellular cytotoxicity at concentrations of 100 g/mL. This led to the activation of apoptotic pathway and augmentation of oxidative stress, whereas the autophagy was recorded to be decreased. At lower concentrations, CD4 expression was decreased and PHA-induced PBMC proliferation was inhibited concurrently with the reverse impact on both activities. Lower doses increased the frequency of CD4+CD25hiFoxp3+ cells as well as other responces such as Th2 (IL-5 and IL-13) and Treg (IL-10). The frequency of T-cells that produce TGF- and IL-10 rose with increasing PoPEx concentrations, especially in CD4+ fraction. Collectively, these findings are suggestive towards the scientific evidences for the immunoregulatory properties of PoPEx on T cells, where these may help to prevent and treat autoimmune and chronic inflammatory illnesses.[19]
- **Moussa Labsi et al. (2016)** they investigate that in vitro treatment of protoscoleces resulted in a PGE dose-dependent substantial reduction in viability. The level of TNF-α and nitric oxide were found to be significantly ($p<0.0001$) decreased in animals, where the cystic echinococcosis-infected animals were treated with PGE. This fall was significantly correlated with a reduction in the expression of CD68 gene particular to pericystic layer of hepatic hydatid cysts and tissue. The rate of hydatid cyst growth inhibition was found to be 63.08% whereas the inhibition of cyst formation was also found to be significant ($p<0.0001$). In the treated mice's liver tissue, iNOS, TNF-a, and NF-kB expression were all significantly reduced ($p<0.0001$). Results show that PGE has immunomodulatory capabilities and an antihydatic scolicidal action, pointing to a possible therapeutic function for it in the treatment of Echinococcus granulosus infection.[20]

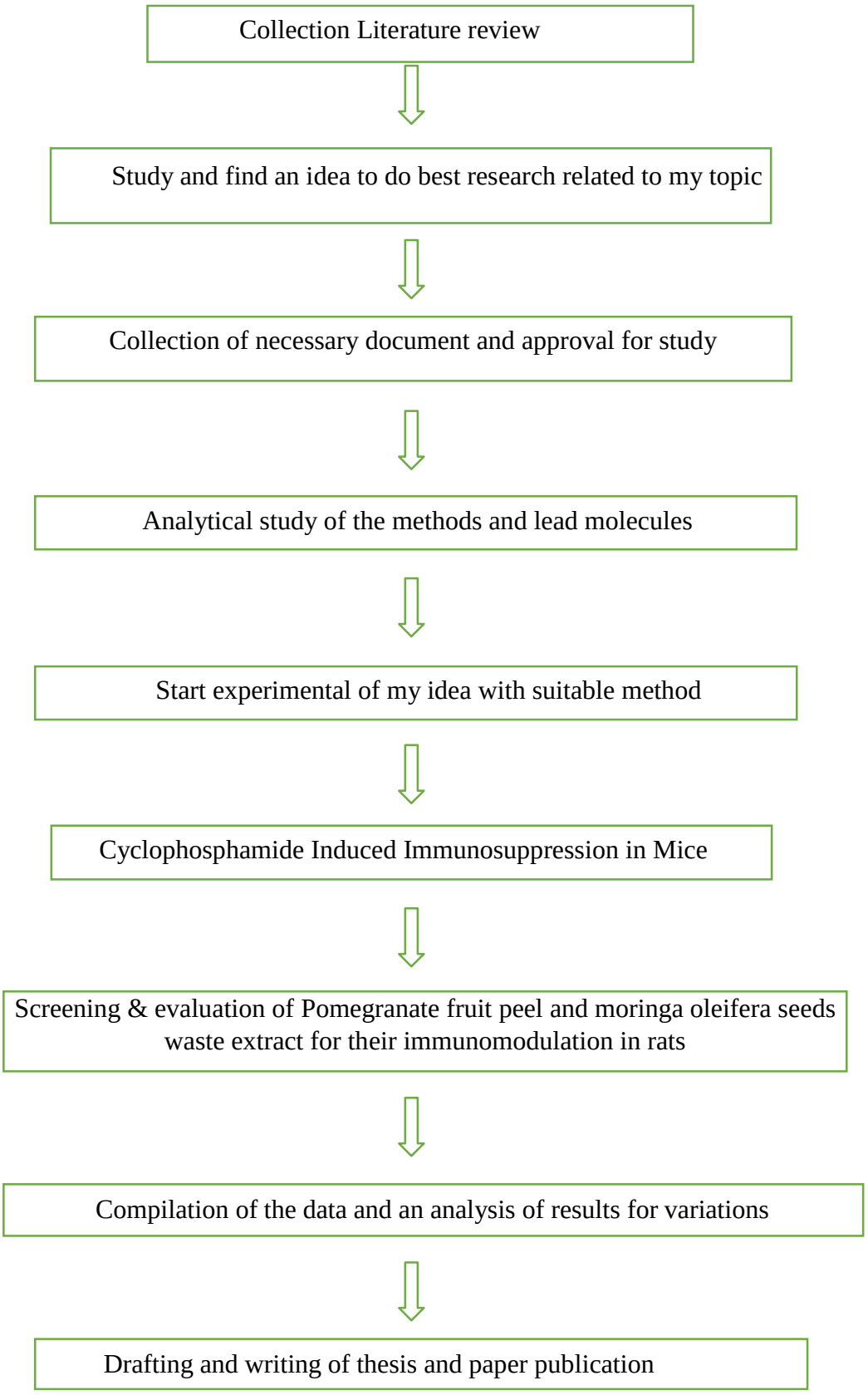
- **I. Stojanovic´ et al. (2017)** they investigated that Punicalin, punicalagin, and ellagic acid were plentiful in the peel extract of pomegranate (PPE), extracted by an aqueous-ethanol solvents system. In this study they have investigated *in vitro* the effect of PPE extracts in animal models of multiple sclerosis and type 1 diabetes (T1D). Interestingly, these extracts were found to have anti-inflammatory effects and antioxidant properties in a synergistic manner. In this study, PPE significantly reduced the level of IL-17, which is reported to be gut-associated lymphoid tissue (GALT) cells produced *in vitro*. This treatment also decreased the level of IL-17 production by activated T cells isolated from animals suffering from autoimmune encephalomyelitis (EAE). This significantly reduced the clinical signs of streptozotocin-induced T1D in C57BL/6 mice and EAE in DA rats. PPE's impact on T1D was mediated by its ability to prevent immune cells from infiltrating pancreatic islets and by interfering with GALT's ability to produce IL-17 and IFN-c. According to our findings, PPE may one day be employed as a phytopharmaceutical to treat several autoimmune and chronic inflammatory diseases.[21]
- **Nfambi et al. (2015)** investigated the effect of methanolic leaf extract of *M. oleifera* on the level of various blood parameters of animals, including a levamisole-positive control group. Animals were treated with doses of 1000 and 500 mg/kg bd wt causes 29.94% and 17.28% reduction in neutrophil adhesion. Mean % increase in the thickness of footpad was found to be 26.9% upon treatment after 8 hours, where the dose of 500 mg/kg bd wt antigen injection in the footpad of rats, and it then decreased to 25.6% after 24 hours. Hemagglutination antibody titer was found to be increased in sheep red blood cells (SRBC), in a dose-dependent manner from 10.730.57 HA units/L, as tested at the dose of 250 mg/kg bd wt. At the dose of 1000 mg/kg bd wt, this was found to be 26.221.70 HA units/L [22].
- **Deshmukh Priyanka et.al (2015)** reported that the aqueous and ethanolic extracts of *Moringa oleifera* having the ability to modulate the immune system in albino rats. There were six groups in this study, each with six rats. Group I functioned as the control group received the food and water normally. In a 45 days study, Group II and IV received the aqueous extract of *Moringa oleifera* at a dose of 400 mg/kg body weight while group III and V were given with oral ethanolic extracts of *Moringa oleifera* at the same dose. Levamisole, a common immunomodulator, was also given orally to Group VI at the standard dose of 50 mg/kg body weight. In comparison to group 1, levamisole, ethanolic extract and hot aqueous extract of *Moringa oleifera* exhibited substantial increase in the HA titre ($P < 0.05$). In the delayed hypersensitivity

test, the volume of paw edema particularly in hot aqueous extract, ethanolic extract treated animals where sheep red blood cells (SRBCs) were significantly increased *(P 0.05)*. (levamisole). As opposed to group I, it was also discovered that the phagocytic index was non-significantly higher in the groups treated with hot aqueous extract, ethanolic extract, and levamisole. As a consequence, the findings demonstrated that Moringa oleifera has immunomodulatory properties.[23]

- **Sandanamudi Anudeep et. al (2016)** they investigated that the proliferation of murine splenocytes and the generation of NO by macrophages were used to measure the immunostimulatory activity of moringa seed resistant protein (MSRP). Splenocyte proliferation was significantly boosted by MSRP at low concentrations (0.01 g/well), compared to poor responses from MSRP at high concentrations. When compared to the control, MSRP caused a 6-fold increase in NO generation, which shows that macrophages had been activated. A strong mitogen, MSRP extracted from defatted moringa seed flour promotes lymphocyte proliferation and triggers macrophage NO production. These results are suggestive for the use of seeds of moringa as a dietary supplement for strengthening host's immune system.[24]
- **Reyes-Becerril et. al. (2021)** they investigated that Vibrio parahaemolyticus and Aeromonas hydrophila were both susceptible to the seed extract's potent antibacterial and antioxidant properties. Fish peripheral blood leukocytes were not subjected to any cytotoxic effects from moringa oleifera seed extract, which also improved immunological functions including respiratory burst activity, nitric oxide generation, superoxide dismutase activity, and catalase activity. In conclusion, MoSE produced using Naviglio's approach was abound in phytochemicals that had strong antioxidant properties, efficient antibacterial activity, and improved immunological parameters in Longfin Yellowtail leukocytes.[25]
- **Coriolano Marilia C. et.al. (2018)** Nitric oxide and the cytokines TNF-, IL-2, IL-6, and IL-10 were released as a result of the action of the water-soluble M. oleifera lectin (WSMoL). The lectin was incubated with peripheral blood mononuclear cells (PBMC), which also resulted in CD8+ T lymphocyte activation. WSMoL encourages immunomodulation in human PBMC, inducing a potential profile for wound healing, and can be tested as an adjuvant in immunosuppressive conditions and wound healing in upcoming in vivo assays.[26]

Plan of Work

The experimental work was planned as-


Collection Literature review
Study and find an idea to do best research related to my topic
Collection of necessary document and approval for study
Analytical study of the methods and lead molecules
Start experimental of my idea with suitable method
Cyclophosphamide Induced Immunosuppression in Mice
Screening & evaluation of Pomegranate fruit peel and moringa oleifera seeds waste extract for their immunomodulation in rats
Compilation of the data and an analysis of results for variations
Drafting and writing of thesis and paper publication

Experimental design

Extraction of peel and seeds will be done by using methanol as a solvent using Soxhlet continuous extraction.

In-Vivo Study-

There will be taken a total of 36 albino mice, weighing between 16 and 21 g. and divided randomly into six group-

Group-1 will be control group with saline treatment

Group-2 will be Negative control group treated with cyclophosphamide 200/kg BW

Group-3 will be Positive control group treated with levamisole hydrochloride 0.68 mg/kg BW

Group - 4 will be Pomegranate peel extract treated group

Group - 5 will be Moringa oliefera seeds extract treated group

Group- 6 will be combined extract treated group treated with mixture of both extract in equal proportion.

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